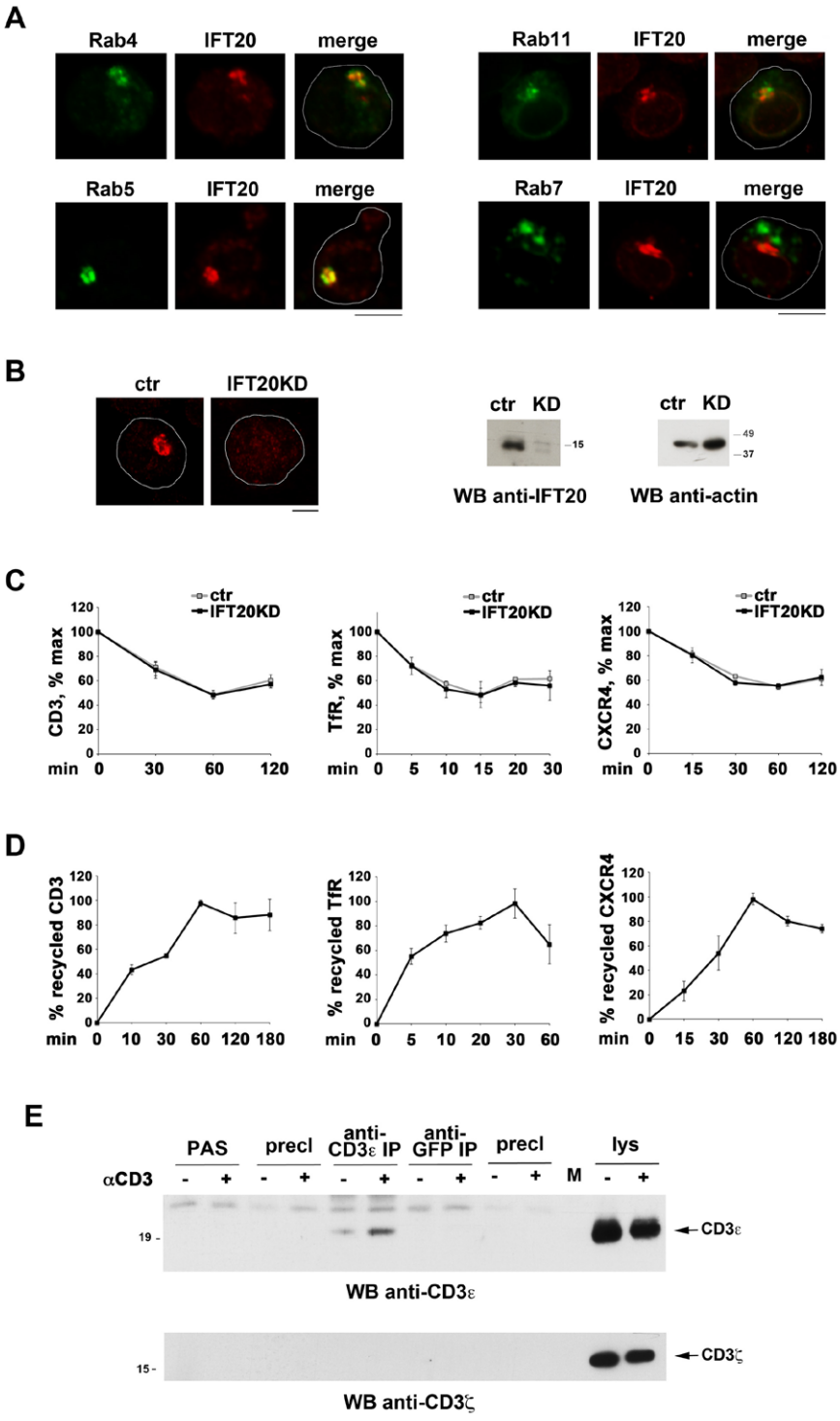

Fig. S1. A. IFT20 colocalization analysis in peripheral T cells and time course of TCR, TfR and CXCR4 internalization and recycling. A. Immunofluorescence analysis of IFT20 in normal peripheral T cells transiently transfected with constructs encoding GFP-tagged Rab4, Rab5, Rab11 and Rab7. Representative images are shown. **B**. Comparison of IFT20 expression in control and IFT20KD Jurkat cells by immunofluorescence and immunoblot (loading control actin). **C**. Flow cytometric analysis of TCR, TfR and CXCR4 internalization in control and IFT20KD Jurkat cells. Cells were incubated on ice with mAb specific for each receptor to allow binding, washed to remove excess mAb (time 0) and shifted to 37°C for the indicated times. The relative levels of receptor were measured by labelling with fluorochrome-labeled secondary antibody both at time 0 (100%) and at each time point after the 37°C shift. The data for each time point refer to duplicate samples from 3 independent experiments. **D**. Flow cytometric analysis of TCR (left), TfR (middle) and CXCR4 (right) recycling in Jurkat cells. The data, which for each time point refer to duplicate samples from 3 independent experiments, are presented as % of the internalized receptors that have recycled to the cell surface. Error bars, SD. **E**. Immunoblot analysis with anti-CD3ε or anti-CD3ζ mAb of CD3ε-specific immunoprecipitates (IPs carried out with OKT3, the same mouse mAb used for activation) from lysates of control Jurkat cells, either non stimulated or activated for 10 min at 37°C by TCR cross-linking. The input lysates (lys), as well as the preclearing samples (precl), no-primary antibody control samples (PAS) and unrelated antibody controls (anti-GFP) were included. Scale bars: 5μm.

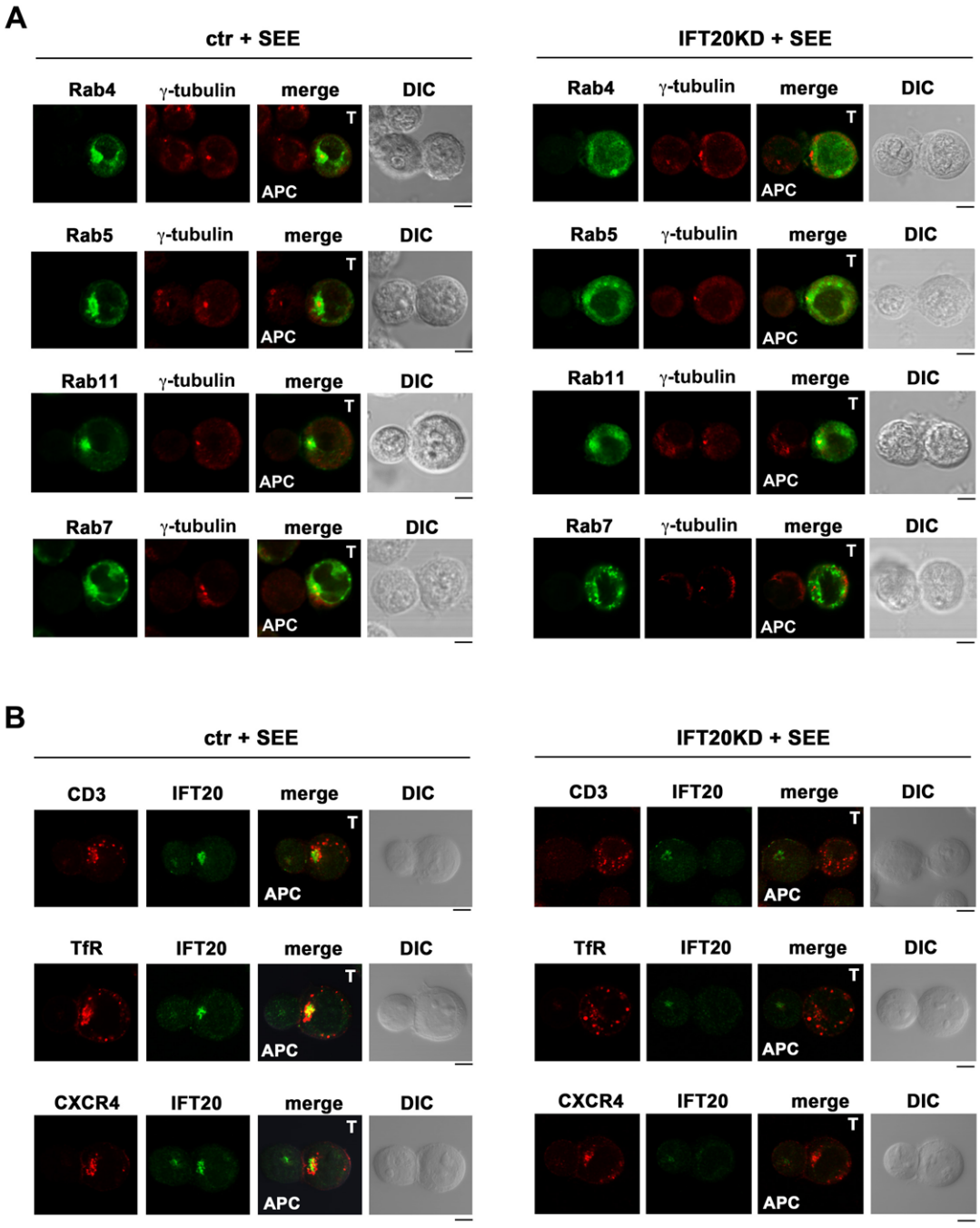

Fig. S2. IFT20 is required for the polarization to the IS of recycling TCR⁺ and TfR⁺ endosomes, but not of CXCR4⁺ endosomes, nor of the MTOC. A. Immunofluorescence analysis of γ-tubulin (centrosome marker) in conjugates of control or IFT20KD Jurkat cells transiently transfected with constructs encoding GFP-tagged Rab4, Rab5, Rab11 or Rab7 and SEE-pulsed Raji cells (APC). Representative images are shown. **B**. Immunofluorescence analysis of recycled TCR, TfR and CXCR4 in conjugates of control or IFT20KD Jurkat cells and SEE-pulsed Raji cells (APC). Cells were incubated with mAb specific for each receptor at 37°C for 2h to allow internalization of receptor:mAb complexes. Residual surface bound mAb was removed by acid stripping. Cells were then mixed with SEE-pulsed Raji cells (APC) and incubated 15 min at 37°C to allow conjugate formation. Receptor:mAb complexes that had undergone polarized recycling to the IS were visualized under permeabilizing conditions using fluorochrome-labeled secondary antibody. Median optical sections are shown. Scale bar: 5 μm.

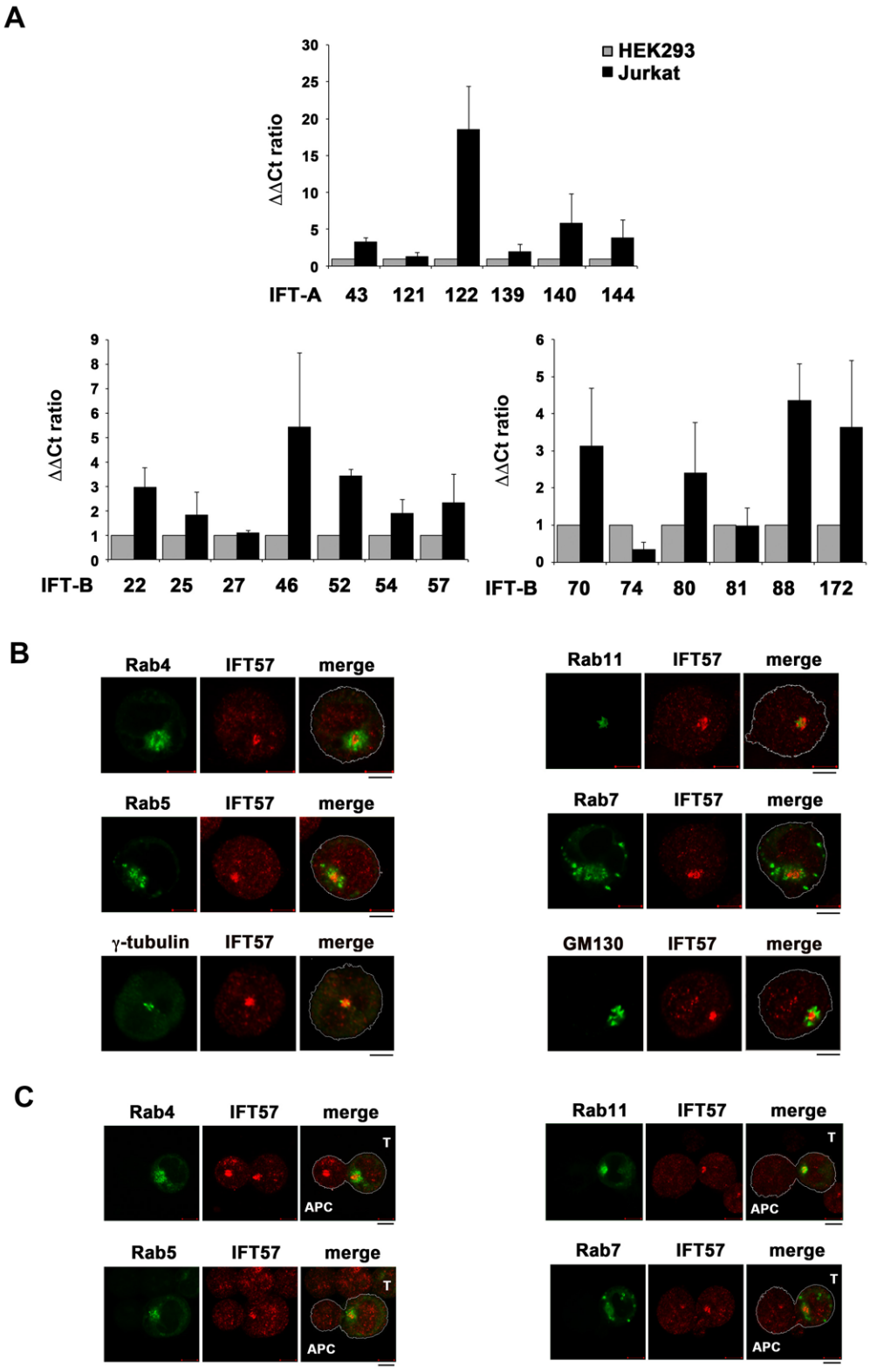

Fig. S3. IFT57 associates with Rab4⁺, Rab5⁺ and Rab11⁺ endosomes and clusters at the IS. A. Quantitative RT-PCR analysis of the transcripts specific for all IFT-A and IFT-B polypeptides in Jurkat T cells and HEK293 cells. The relative abundance of gene transcripts was determined on triplicate samples using the ddCt method and was normalized to HPRT1. The data (mean±SD; n≥3) are expressed as normalized fold expression in Jurkat cells vs HEK293 cells (set for each IFT component as 1). **B.** Immunofluorescence analysis of IFT57 localization in Jurkat cells stably transfected with expression constructs encoding GFP-tagged Rab4, Rab5, Rab11 and Rab7, either as such (**B**) or following conjugate formation with SEE-loaded Raji cells (APC) (**C**). The third row in panel B shows Jurkat cells co-stained with anti-IFT57 and either anti-γ-tubulin (centrosome marker, left) or anti-GM130 (Golgi marker, right) antibodies. Median optical sections are shown. Scale bar: 5 μm.

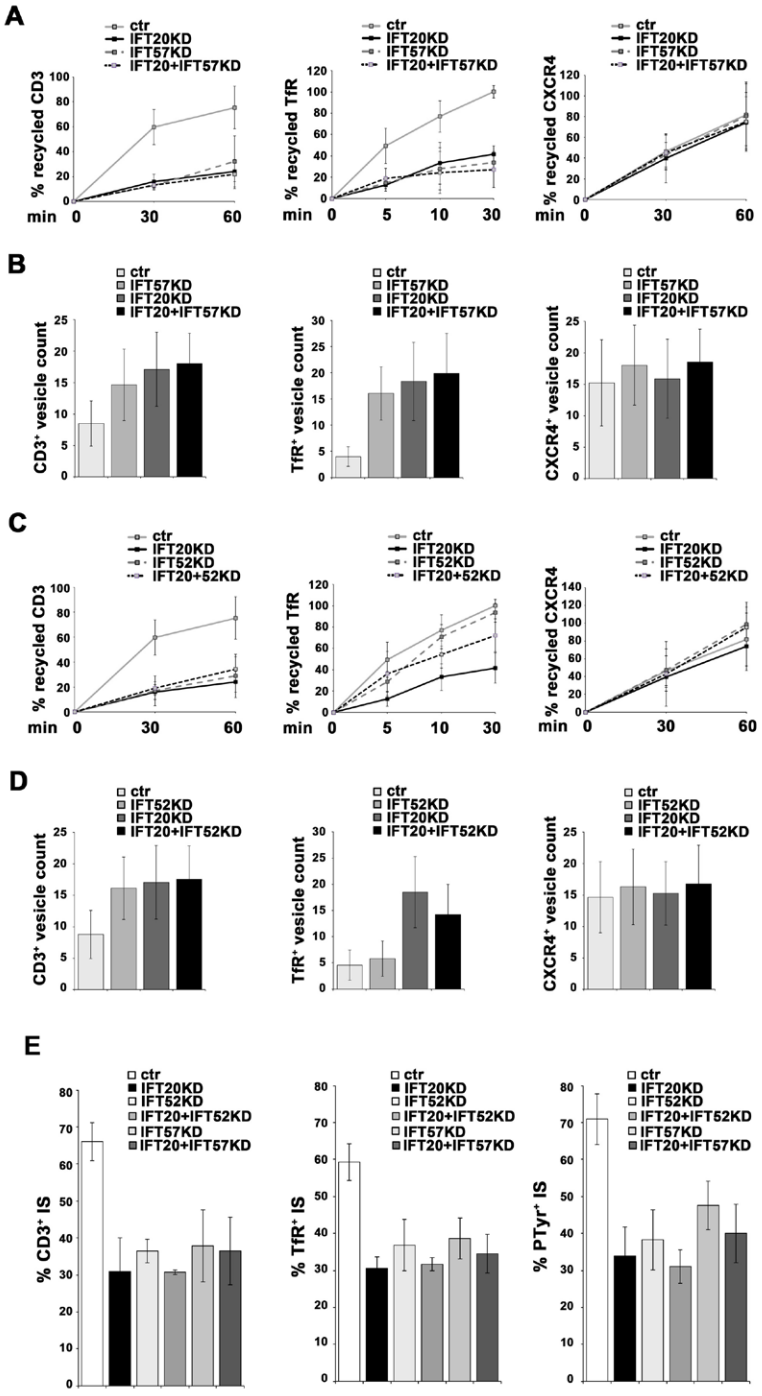

Fig. S4. IFT20, IFT57 and IFT52 participate in the same pathway of TCR and TfR receptor recycling. A, C. Flow cytometric analysis of TCR (left), TfR (middle) and CXCR4 (right) recycling in control and IFT20KD Jurkat cells transiently knocked down for IFT57 (**A**) or IFT52 (**C**) expression using specific siRNAs (~69% of IFT52 RNAi and ~38% of IFT57 RNAi in control Jurkat cells, ~63% of IFT52 RNAi or ~53% of IFT57 in IFT20KD Jurkat cells). RLUC siRNAs were used as control. A construct encoding GFP under the control of a constitutive promoter was included in each transfection as control. Recycling was analyzed 24 h post-transfection, gating on GFP$^+$ live cells. The data, which for each time point refer to duplicate samples from 3 independent experiments, are presented as % of the internalized receptors that have recycled to the cell surface. **B, D.** Counts of vesicles containing internalized CD3 (left), TfR (middle) or CXCR4 (right) in Jurkat cells and IFT20KD cells transiently knocked down for IFT57 (**B**) or IFT52 (**D**) expression using specific siRNAs. Cells were incubated with saturating concentrations of mAb specific for each receptor at 37°C for 2 h, fixed and permeabilized, and internalized receptor:mAb complexes were labeled using fluorochrome-labeled secondary antibody and visualized by confocal microscopy. The data are presented as number of labeled vesicles in individual medial confocal sections. At least 20 cells were analyzed for each receptor. Representative images from 3 independent experiments are shown. Scale bar: 5 μm. Error bars, SD. **E.** Results of immunofluorescence analyses of TCR, TfR and PTyr in conjugates of SEE-pulsed Raji cells (APC) and control or IFT20KD Jurkat cells transiently knocked down for IFT57 or IFT52 expression using specific siRNAs. RLUC siRNAs were used as control. Cells were processed for conjugate formation 24 h post-transfection. The histograms show the percentage of conjugates with TCR and TfR polarization at the IS, or of conjugates harboring PTyr staining at the IS. The measurements were taken on at least 300 conjugates from 3 independent experiments. Error bars, SD.

Table S1. List of the primers used in this study

	Forward 5'-3'	Reverse 5'-3'
WDR19 (IFT144)	CTG TGT GGC CAA TAT TCA CG	GCT GAT TGG TCA GCA GTT CA
IFT140	GAC TGG AGA AGT GAC GGT GT	CAC AAG ACC AAG ACA CCA AG
THM1 (IFT139)	GAG CAA AAT TGG AAC CAG GA	TCA CGA TCT TTC CGA GCT TT
IFT122	AGA TGA GGA CCC GTT CAC	GTG AGC GGA AGT ATT GCC
WDR35 (IFT121)	TCG CAG TAT GAG CTG GAA TG	TAC GAT TGC CAT CCA CTG AA
IFT43	TCG CTA CAG CTT GGC TAC CT	AGG AAT TCT TGC CAT TGA GG
IFT172	AGT TGA CGT ATG TGG GAC CT	GTG AGC CAC CAA GTA ACG
IFT88	ATA GAT GCC TCC TAT GTG GAC	CTG GAA GCA AAT CAT CTC C
IFT81	TGG ACA GTC TCA ATA AGG AGC	TCT CTG ATA TCC ACA AGT TGC T
IFT80	CAG TTG GAG AAG ATG GAC AA	GAG GTT TAA TGA TTA GCT GCT TG
IFT74	GAC AGC AAG ACC AGG TTC TC	TCA TTC CAG TCA AAC CTT GT
TTC30A (IFT70)	GTC GCT GCT AGG CTA CTG CT	AGG CGG TAC TGC TCC AGT T
IFT57	CAT GTT CGT GGT GAT GGA	ACA TGT AGA ACT GTT CGC CAG
IFT54	AGC AGC TGA TCA AAG ACC A	TGT TCA CCT TCT CGA AGT CA
IFT52	CGG AGT AAT TTG GAA GAT TCA	ATC TCC ACC AGT GTC AAG AT
IFT46	TGA GCA TTT GCC AGT TTC TG	TCA GTT TGT GGT CCA GGT CA
IFT27	CAG ACG AGC AGT GGA CTC AG	GAA AGG GGC TTC GAA GTT TT
IFT25	GAA ATT GTG GCA CAT GAT GG	TCT GCA GAA ACG CTA TGC AC
RABL5 (IFT22)	AAA CCA GGC TCT GGA GAT GA	GTT TGA GTG CAC CAG CTT CA
HPRT1	AGA TGG TCA AGG TCG CAA G	GTA TTC ATT ATA GTC AAG GGC ATA TC